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MASS PROPAGATION OF *BOUGAINVILLEA SPECTABILIS* THROUGH SHOOT TIP CULTURE

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Abstract

A simple and efficient *In vitro* regeneration protocol for *Bougainvillea spectabilis* Willd., was developed from shoot tips of 5 year old plants. Shoot tips were surface sterilized and cultured on MS medium supplemented with different concentrations of BAP (0.25 – 2.0mg/l) or Kinetin (0.25 – 2.0mg/l) and NAA (0.1-0.5 mg/l) in combination with BAP (0.25 – 5.0 mg/l). It was observed that BAP (0.25mg/l) combined with 0.1mg/l NAA gave best results where 90% shoots were developed into plantlets. Best multiple shoot formation was recorded on MS medium supplemented with 1.0mg/l BAP and 250 mg/ l glutamine.

The regenerated shoots were successfully rooted on half strength MS medium with different concentrations and combinations of auxin. It was observed that 2.5 mg/l NAA combined with 2.5mg/l IBA gave 100% root induction. The plantlets after weaning and acclimatization were transferred to pots and then to soil for establishment.

Introduction

In recent years, propagation of numerous ornamental plants by tissue culture has become an accepted commercial practice (Frankenberger *et al.*, 1981).The current Micropropagation of plants have allowed for strong and continued growth within the micro propagation industry. Bougainvillea (*Bougainvillea spectabilis* Willd) belongs to the family Nyctaginaceae. The family has 30 genera and 300 species. In Pakistan it is represented by 5 genera and 11 species. It is native to Latin America (Brazil), commonly grown in gardens, porches and boundary walls. Flower remains throughout the year particularly from April to August. The propagation of Bougainvillea is difficult because in our climatic conditions it does not produce seeds while from cutting the percentage of success is low and cumbersome. Some cultivars are difficult to root and the leafy cuttings need mist condition after treatment with root promoting hormone (Hartman & Kester, 1989). Tissue culture techniques have been successfully employed to produce large number of difficult to propagate plants as reported by Khan *et al*., (1985) and Javed *et al*., (1996). Micropropagation is rapid, efficient and plants can be produced year round for the market by this technique. Micropropagation of Bougainvillea by shoot tip culture has been successful (Chaturvedi *et al.*, 1978). Keeping in view the economical, aesthetic and ornamental value, these studies were carried out to improve the micropropagation protocol of Bougainvillea through shoot tip culture for commercialization.

Materials and Methods

Shoot tips of Bougainvillea Cv. Texans Dawn were obtained from 5 year old plants growing outside the lawn of NIFA campus. One cm long shoot tips of Bougainvillea were treated with 3 drops of Zip as a detergent and then washed by running tap water for

½ an hour to remove dust particles. The explants were surface sterilized with 70% ethanol for 30 seconds followed by 0.05% Mercuric Chloride ($HgCl_2$) for 5 minutes on shaker according to the procedure of Zamir *et al.,* (2004). Then the shoot tips were rinsed three times with sterile distilled water in Laminar flow bench to remove sterilants.

MS (Murashige & Skoog 1962) basal medium supplemented with cytokinins and auxins and 3% sucrose as a carbon source was used. The medium was solidified with 0.7% plant agar. The pH of the medium was adjusted to 5.7 before adding agar and autoclaved at 121 ^oC, 1.1 kg / cm^2 for 25 minutes. The cultures were kept in growth chambers at 26 ± 1^oC under a 16hrs light/8hrs dark with a light intensity of 2000-3000 lux. Experiment was arranged in a randomized complete block design with three replications per treatment, each with 20 explants. The data were recorded after 5-7 weeks and statistically analyzed by using Duncan's Multiple Range Test (Steel & Torrie, 1980), to check the level of significance between the treatments.

When shoot tips developed into plantlets, they were sub-cultured on MS medium supplemented with different concentrations of BAP (0.5, 1.0 and 2.0 mg/l) alone or in combination with glutamine (250 and 500mg/l). The cultures were incubated for a period of 7 weeks.

In order to induce root, shoot with excellent growth, having 4-5 leaves were transferred to MS medium with different concentration and combinations of IAA, IBA and NAA. The complete rooted plantlets were transferred into pots containing a mixture of sand + silt (1:1) and compost after washing the roots thoroughly to remove any remains of the medium. In the first week of transfer the potted plants were covered with glass beaker to maintain high humidity and then to soil for establishment. Most of the plants flowered within 3-4 months.

Results and Discussion

Effect of kinetin, BAP and BAP combined with NAA on shoot development: Shoots development varied with the different concentration of kinetin (0.25, 0.5, 1.0 and 2.0 mg/l), BAP (0.25, 0.5, 1.0 and 2.0 mg/l) alone or BAP (0.1, 0.25, and 0.5 mg/l) with NAA (0.1, 0.25 and 0.5 mg/l) supplemented to MS medium. The best results were found with 0.25 mg/l BAP combined with 0.1 mg/l NAA and total of 54 plants out of 60 explants (90%) were produced (Table 1). Remaining concentration 0.25 mg/l BAP + 0.25 mg/l NAA, 0.5 mg/l BAP + 0.25 mg/l NAA and 0.1 mg/l BAP when combined with 0.1 mg/l NAA produced 45, 44 and 37 plants respectively. Javed *et al*., (1996) found best results with 0.25 mg/l BAP and 0.25 mg/l NAA followed by either 0.25 mg/l BAP and 0.1 mg/l NAA or 0.5 mg/l BAP and 0.25 mg/l NAA respectively where 40, 35, and 34 plants were produced. Only 20 % plants developed in MS control medium having no phytohormone. The kinetin and BAP alone at concentration 1.0 mg/l and 0.25 mg/l gave rise to 37 and 36 plants, respectively (Table 1). Khan *et al.,* (2004) also observed highest profuse adventitious shoot in *Cordyline terminalis* cultured on medium containing a combination of Kinetin at 4.0 mg/l and NAA at 0.5 mg/l. However our concentrations of Kinetin were much less as compared to above. A maximum increase of 6 times in plant height was obtained as compared to the original shoot length when combination of 0.25 mg/l BAP and 0.1 mg/l NAA were used (Fig. 1A). Sajid *et al.,* (2006) also observed that NAA was more effective for promoting shoot length in case of grapes germplasm.

Table 1.Effect of different concentrations of BAP or Kinetin alone or BAP with NAA on development of plantlets and callus formation of *Bougainvillea spectabilis* cultured on MS medium

Treatments (mg/l)	No. of cultured shoot	No. of shoot developed into plantlets	Developed plantlets %	Average plantlet height (cm)	Callus formation
BAP					
0.25	60	36 a	60	1.33 bc	+
0.5	60	25 ab	41.6	1.60 ab	-
1.0	60	17 b	28.3	1.78 a	-
2.0	60	26 ab	43.3	0.90 d	+
Control	60	10 c	16.6	0.52 d	-
Kinetin					
0.25	60	32 a	53.3	0.51 c	-
0.5	60	10 b	16.7	0.64 bc	-
1.0	60	37 a	61.7	1.11 a	+
2.0	60	26 a	43.3	0.48 c	+
Control	60	5 b	8.3	0.90 ab	-
NAA+ BAP					
0.1 + 0.25	60	54 a	90	1.95 b	+
0.25 + 0.25	60	45 b	75	2.40 a	+
0.5 + 0.25	60	29 cd	48.3	0.83 c	+ +
0.1 + 0.5	60	28 cd	46.6	2.00 b	+
0.25 + 0.5	60	44 b	73.3	1.66 b	+ +
0.5 + 0.5	60	19 de	31.6	1.76 b	+ + +
0.1 + 0.1	60	37 bc	61.6	1.70 b	+
0.25 + 0.1	60	28 cd	46.6	0.86 c	+
0.5 + 0.1	60	32 cd	53.3	1.73 b	+ +
Control	60	12 e	20	0.65 c	-

Means followed by different letters within column are statistically different at 5% level of significance, using DMR test.

Callus formation: - No callus, + Little callus, ++ Moderate callus, +++ Profuse callus

Table 2. *In vitro* micropropagation of *Bougainvillea spectabilis* on MS medium containing different concentrations and combinations of BAP and glutamine.

BAP + Glutamine (mg/l)	No. of cultured plantlets	Total No. of shoots developed	Average No. of shoots/ plantlets	Range of shoots/ plantlet
0.5 + 250	60	205 b	4.31	1-6
1.0 + 250	60	260 a	5.41	2-6
2.0 + 250	60	140 c	2.33	2-3
0.5 + 500	60	138 c	2.30	1-3
1.0 + 500	60	150 c	2.50	2-4
2.0 + 500	60	120 c	2.00	1-3

Means followed by different letters within column are statistically different at 5% level of significance, using DMR test.

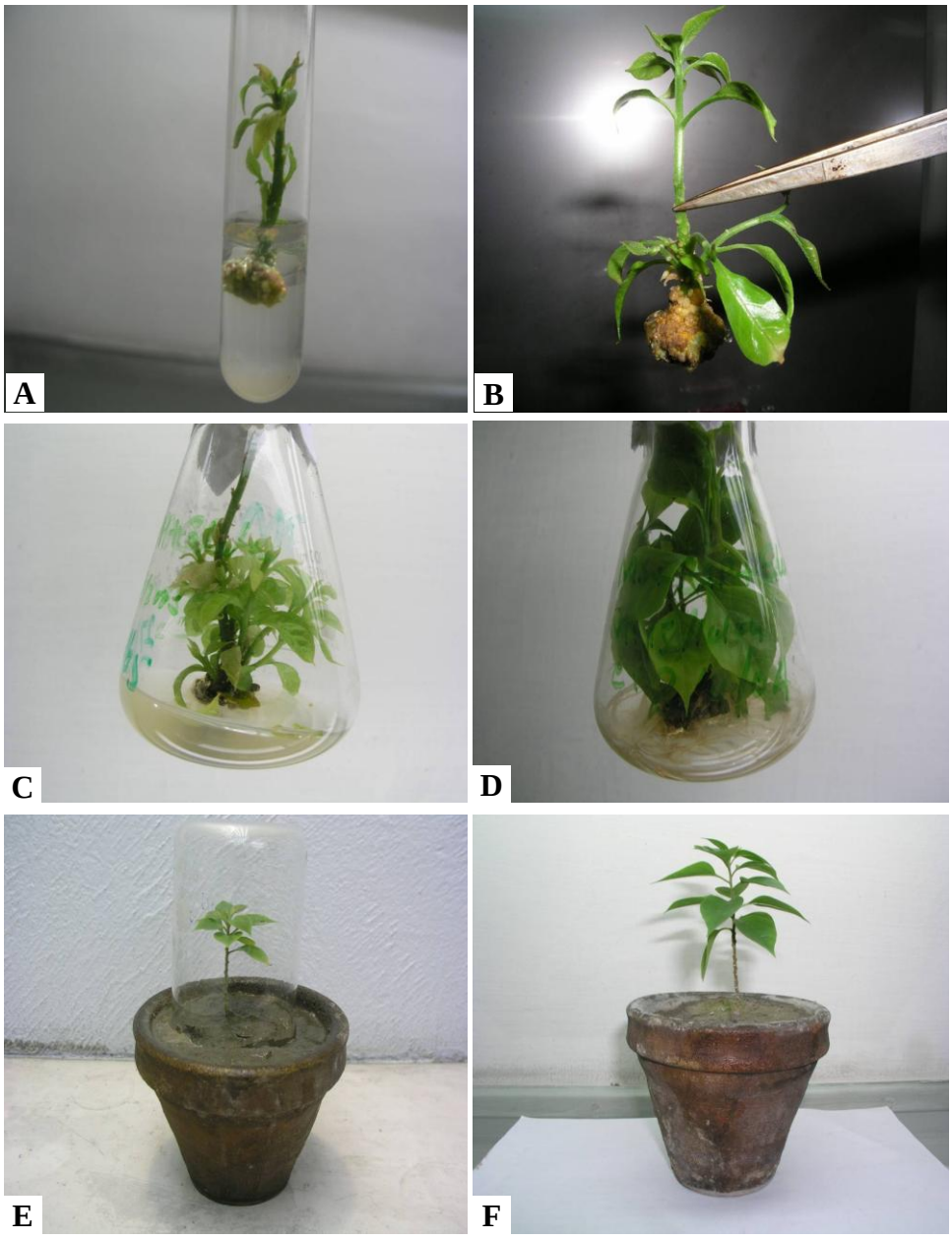

Fig. 1. A. Shoot proliferation/callus indication in Bougainvillea on 0.25 mg/l BAP and 0.1 mg/l NAA; B. Callus formation at the base of shoot on 0.5 mg/l BAP and 0.5mg/l NAA; C. A clump of *in vitro* multiple shoots with callus at the basal part on 1mg/l BAP and 250 mg/l glutamine; D. *In vitro* root induction on medium containing 2.5 mg/l IBA and 2.5 mg/l NAA; E to F. Acclimatization of plantlets in pots.

Table 3. Effect of different auxins alone or in combination on rooting of *Bougainvillea spectaibis* shoot when added to half MS medium.

Auxins (mg/l)	No. of cultured plantlets	No. of plantlets rooted	Percent plantlets rooted	Ave. No. of roots/ plant	Ave. Root length (cm)
IAA 2.5	60	15 c	25	2.7 e	2.35 a
IBA 2.5	60	48 ab	80	15.5 c	2.55 a
NAA 2.5	60	18 c	30	7.5 d	1.60 b
IAA 2.5 + IBA 2.5	60	36 b	60	5.0 de	1.40 bc
IAA 2.5 + NAA 2.5	60	12 c	2	25.0 b	1.10 cd
NAA 2.5 + IBA 2.5	60	60 a	100	23.0 b	0.75 de
IAA 5.0 + IBA 5.0	60	45 b	75	12.5 c	1.75 b
IAA 5.0 + NAA5.0	60	42 b	70	30.0 a	0.53 e
NAA 5.0 + IBA 5.0	60	39 b	65	22.9 b	0.48 e
Control	60	6 c	10	4.3 de	1.53 b

Means followed by different letters within column are statistically different at 5% level of significance, using DMR test.

Callus induction: Cytokinin greatly affected the intensity of basal callusing (Amin & Jaiswal 1993). In the present study, 0.5 mg/l BAP and 0.5mg/l NAA gave big callus at the base of explant (Fig. 1B) while 0.1mg/l BAP and 0.5 mg/l NAA and 0.25 mg/l BAP and 0.5 mg/l NAA gave moderate one (Table 1). Hassan *et al*., (2000) obtained best calli in leaf explant of Kiwi fruit when 1mg/l and 2mg/l 2, 4 D were used in combination with 0.5 mg/l BAP. Khan *et al.,* (2004) also preferred callus culture to shoot tip culture as it maximizes the shoot multiplication and make it a feasible protocol.

Proliferation of shoots: BAP at 1mg/l with glutamine 250 mg/l induced significantly higher number of multiple shoots (260) where on an average of 5.41 new shoots per plant were produced (Table 2, Fig. C). The treatments comprising 0.5 mg/l BAP and 250 mg/l glutamine produced only 205 shoots having an average of 4.31 shoots/ plant while the other concentration gave no significant enhancement in shoot multiplication and number of shoots per plant. These results are in confirmity with the results of Zamir *et al*., (2003), who reported highest numbers of shoots in guava shoot tips cultured on MS medium supplemented with 1 mg/l BAP and 250 mg/l glutamine. Similar observation has been recorded by Khan *et al.,* (2004) who cultured *Ixora coccinea* plants for 12 weeks on BAP with Peptone. But the concentration of BAP used was very low i.e., 0.005, 0.05 and 0.5 mg/l as compared to our concentration of BAP. Sajid *et al.,* (2006) also observed poor shoot proliferations in grapes germplasm when low levels of BAP and auxins were used. However, when the BAP concentration was increased beyond 0.3mg/l there was an increase in the number of shoots per plant.

Effect of different concentration of auxins on root development: The highest numbers of plants (100%) were rooted when half strength MS medium was supplemented with 2.5mg/l IBA and NAA 2.5 mg/l (Table 3, Fig. D). Other treatments like 2.5 mg/l IBA alone and 5 mg/l IBA and 5 mg/l IAA or 5 mg/l NAA and 5 mg/l IAA when added to the medium showed significantly higher percentage (80, 75 and 70) of rooted plantlets (Table 3). The combination of 2.5 mg/l IBA and 2.5 mg/l NAA proved superior for root induction of Bougainvillea. Our results are in line with the results of Sinha *et al.,* (1997) who also got 100% rooting in *Bougainvillea buttiana* cultured on half strength MS

medium supplemented with 1.0 mg/l each of IBA and NAA. Swamy & Sahijram (1998) obtained 47.6 % rooting in Bougainvillea when 5 mg/l IBA was added to MS medium. Javed *et al*., (1996) also found significantly higher percentage of root development (70, 60 and 67.5) when 5 mg/l IBA and 5 mg/l NAA and either 2.5 mg/l NAA or 5 mg/ l alone was supplemented to the medium. Misra & Datta (1999) also suggested half concentration of salts for root induction in *Ixora*. Similar observations has been made in *Ixora singaporensis* by Malathy & Pai (1998) in which roots were induced by transferring the shoots previously cultured in MS basal to B5, a lower salt medium.

The *In vitro* growth response of Bougainvillea under the study has strengthened our under standing on culture establishment, rooting and shoot proliferation which are vital in the commercial propagation. From this protocol, supply of both rooted plantlets and proliferating culture of shoots will be available continuously, which are essential requirements for mass propagation (Rajaseger, 1999).

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References

Amin, M.N and V.S. Jaiswal. 1993. *In vitro* response of apical bud explants from mature trees of jackfruit (*Artocarpus heterophyllus* Lam). *Plant Cell, Tissue and Organ Culture*, 33: 59-65.

Chaturvedi, A., K. Sharma and P.N. Prasad. 1978. Shoot apex culture of *Bougainvillea glabra* Magnifica. *HortScince*, 13: 36.

Frankenberger, E.A., P.M. Hasegawa and E.C. Tigchelaar. 1981. Influence of environmental and developmental state on the shooting capacity of Cordyline genotypes. *Plant Physiol*., 102: 221-232.

Hartman, H.T. and D.I. Kester. 1989. *Plant Propagation, Principles and Practices*. 4th edition. Prentice Hall of India Private Limited, New Delhi.

Hassan, S., R.Zamir and M.Tariq. 2000. Micro propagation of Kiwi fruit (*Actnidi chinensis*) through leaf callus culture. *Pakistan J. Agric. Res*., 16(1): 31-34.

Javed, A.M., H.Said and N.Saima. 1996. *In vitro* propagation of *Bougainvillea spectubilis* through shoot apex culture. *Pak. J. Bot*., 28: 207-211.

Khan, M.A., M. Ghamdi and M. Jahjh. 1985. *In vitro* propagation of date palm (*Phoenix dactylifera* L.). *Pakistan Journal of Agriculture Science*, 22: 177-180.

Khan, S., M. Iftikhar and B. Saeed. 2004. An economical and efficient method for mass propagation of *Ixora coccinea*. *Pak. J. Bot*., 36(4): 751-756.

Khan, S., S. Naz and B. Saeed. 2004. *In vitro* production of *Cordyline terminalis* for commercialization. *Pak. J. Bot.*, 36(4): 757-761.

Malathy, S and J.S. Pai. 1998. *In vitro* propagation of Ixora singaporensis. *Curr. Sci*., 75: 545-547.

Misra, P and S. K. Datta. 1999. Propagation of Ixora using low salt media. *Curr. Sci*., 77: 1138-1140.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*., 15: 473-497.

Rajaseger, G., H.T.W. Tan, I.M. Turner, L.G. Saw and P.P. Kumar. 1999. Analysis of Peninsular Malaysian Ixora species and selected population and mutants by RAPD. *Annals of Botany*, 84: 253-257.

Sajid, G.M., M.K. Ilyas and R. Anwar. 2006. Effect of diverse hormonal regimes on in vitro growth of grape germplasm. *Pak. J. Bot.*, 38(2): 385-391.

Sinha, P., A. Rahman and S.K. Roy. 1997. Micropropagation of *Bougainvillea buttiana* Hol & Sandley. *Plant tissue cult*., 7(2): 117-124

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedure of Statistics. McGraw Hill, New York.

Swamy, D.R. and L. Sahijram. 1998. Tissue culture propagation of Bougainvillea. *Gartenbauwissens Chaft,* 53: 174-176.

Zamir, R., G.S.S. Khattak, T.Muhammad, S.A. Shah, J.A. Khan and N. Ali. 2003. *In vitro* mutagenesis in guava (*Psidium guajava* L). *Pak. J. Bot.*, 35: 825-828.

Zamir, R., Syed Tariq Shah, Nawab Ali, G.S.S. Khattak and T. Muhammad. 2004. Studies on *in vitro* surface sterilization and antioxidants on guava shoot tips and nodal explants. *Pak. J. Biotech*., 1(2): 12-16.

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